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CHAPTER 9

SERIAL MICROBIOTA ANALYSIS AFTER FECAL MICROBIOTA TRANSPLANTATION IN A CHILD WITH DOWN SYNDROME

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ABSTRACT

Fecal microbiota transplantation (FMT) is a very effective treatment for recurrent *Clostridium difficile* infection (CDI) in adults. However, there is a paucity of data on FMT in children and associated microbiome changes in this specific group. We describe a child with Down syndrome and intracranial malignancy, who received FMT for recurrent CDI. Detailed microbiota analysis prior to and following FMT, and pre- and post-recurrence, linked to microbial communities in the donor feces showed that the patient developed a unique microbiota profile after FMT which was very stable over time despite CDI recurrence and subsequent fidaxomicin therapy. *Bacteroidetes* were stably acquired from donor feces, while *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* and *Proteobacteria* were unique to the patient. The diversity of microbiota of our patient increased from a Shannon diversity index of 2.08 pre-FMT to 3.12 post-FMT.

Our findings underscore that patients with Down syndrome, with an alternate immune system, may well tolerate and benefit from FMT even in a severe immunocompromised state.

INTRODUCTION

Clostridium difficile is an obligate anaerobic, Gram-positive, spore-forming bacillus, and the most frequent cause of infectious healthcare associated diarrhea. Infection commonly results from prolonged administration of antibiotic therapies which disrupts the intestinal microbiota composition, facilitating colonization with *C. difficile*.¹ Current treatment protocols recommend metronidazole (30 mg/kg/day for 10 days) as initial treatment option for pediatric *C. difficile* infection (CDI). However, over 20% develop a recurrent infection.^{2,3} Risk factors for the development of recurrent CDI in children include underlying inflammatory bowel disease, malignancy, recent surgery, and the number of antibiotic exposures by class.^{2,4-6} Fecal microbiota transplantation (FMT) has been proven a safe and effective treatment for recurrent CDI in adults and children.⁶⁻⁹ However, pediatric studies are limited in sample size, and there is a paucity of data on the associated microbiome changes in this specific group.⁶ Here, we describe a 14 year old child with Down syndrome and intracranial malignancy, with recurrent CDI treated with FMT. Furthermore, we describe the impact of FMT on the gut microbiota composition, prior to and following FMT, linked to microbial communities in the donor feces.

CASE PRESENTATION

A 14-year old girl was referred for FMT because of recurrent CDI. Her medical history revealed Down syndrome and a recently diagnosed choroid plexus carcinoma. Total surgical resection of the choroid plexus carcinoma was performed. Postoperatively, the patient was treated with alternating cycles of cyclophosphamide/etoposide/vincristine and carboplatin/etoposide/vincristine. A magnetic resonance imaging scan performed after two treatment cycles showed continued complete response. After

two cycles of chemotherapy, cranio-spinal radiotherapy was administered for a period of six weeks. During this period, she was treated with ciprofloxacin because of a pharyngeal infection with *Pseudomonas aeruginosa* and trimethoprim-sulfamethoxazole was prescribed as *Pneumocystis jirovecii* pneumonia prophylaxis. At this stage, the patient developed diarrhea up to four times per day. Stool tests for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, parasites and adeno- and rotavirus were all negative.

After one month of diarrhea, these tests were repeated and extended with toxigenic culture for *C. difficile*, which was positive. This first episode of CDI was treated with a 14-day course of oral metronidazole (500mg TID). Her gastrointestinal complaints resolved completely during this course but reoccurred several days after cessation of CDI therapy (*C. difficile* toxin positive), which was treated with a 14-day course of metronidazole (500mg TID). Again, watery diarrhea resolved completely but reoccurred within one week after cessation of antibiotics, and the patient was now treated with a 14-day course of oral vancomycin (250mg TID). Shortly after cessation of vancomycin, patient developed watery diarrhea again (*C. difficile* toxin positive), and treatment with vancomycin was restarted for a prolonged period of four weeks.

Due to the recurrent/refractory nature of CDI, the ongoing low blood counts, and poor clinical condition (immobility, lack of energy, complete tube feeding dependency) it was decided, in consultation with her parents, to renounce from further chemotherapy, two cycles prior to completion of the protocol. Since administration of antibiotics seemed to control but not clear CDI, and because of the great impact of the infection on her general well-being, treatment with FMT was suggested. The child's parents agreed to this therapy, and informed consent was obtained (including collection of follow-up fecal samples). FMT material was provided by OpenBiome, an international public stool bank.¹⁰ The donor feces (adult donor) solution was administered via a nasoduodenal tube under general anaesthesia,

preceded by five days of oral vancomycin (250 mg TID), and full bowel lavage. No medication was prescribed after FMT. In the first five days following FMT, patient did not pass any stools. In the subsequent two weeks the diarrhea had resolved completely and she passed formed stools twice a day.

Unfortunately, the patient developed diarrhea again 18 days after FMT and cultures were positive for *C. difficile*. Since it has been suggested to treat a first recurrence of CDI after FMT with antibiotics, with a preference for the narrow spectrum antibiotic fidaxomicin¹¹, a 10-day course of fidaxomicin 200mg (BID) was prescribed. With this policy, gastro-intestinal symptoms resolved completely, stool consistency normalized and the general well-being of the patient improved to pre-CDI level. No long-term side effects (e.g. auto-immune disease, diabetes) were reported.

Microbiota analysis

Intestinal microbiota analysis was performed on fecal samples of the patient, collected before and after FMT (Figure 1). Microbiota profiles of the patient were compared with the microbial communities of the donor. Microbiota analysis was performed by the interspace microbiota profiling technique (IS-pro). The IS-pro technique is an alternative to sequencing, which allows detection of bacterial DNA and its taxonomic classification down to species-level based on the 16S-23S ribosomal interspace fragment length.^{12,13} The procedure is suited for routine diagnostic use, and results are available within hours.^{13,14} Pre-FMT microbiota composition of our patient was characterized by very low diversity (Shannon diversity index 2.08); only a limited number of different species was present in low abundance. A sample taken five days post FMT (first stool) showed a marked increase in diversity (Shannon diversity index 3.12) compared to pre-FMT, which was mainly attributable to species from the phylum *Bacteroidetes*. The transfer of the phylum *Bacteroidetes* was highly efficient: almost all *Bacteroidetes* species present in the donor feces were also found in the recipient five days after FMT and remained present in all following time points.

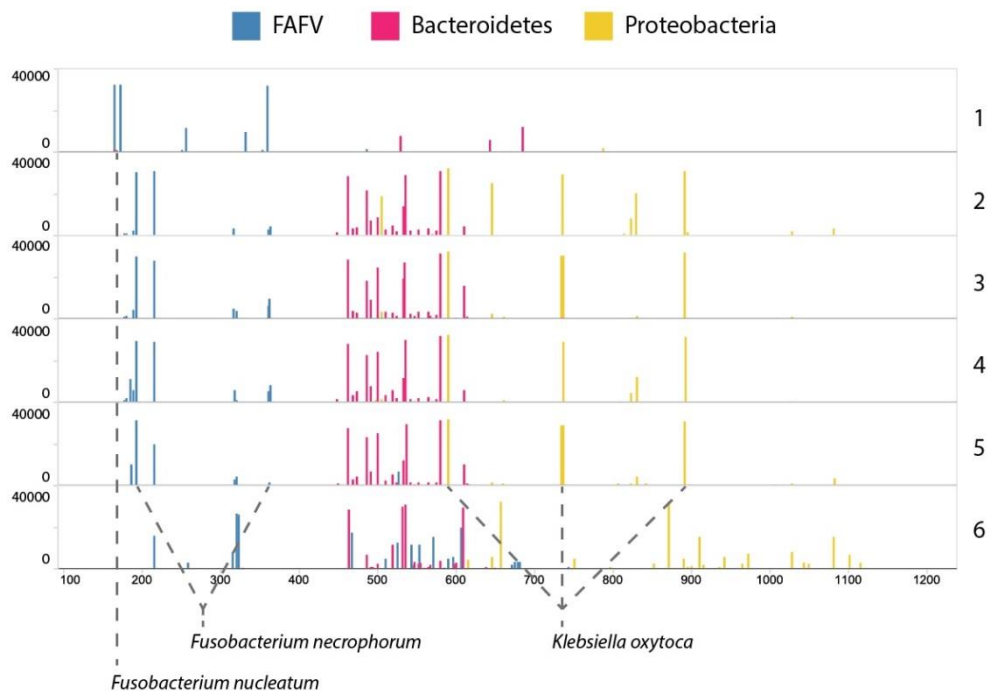


Figure 1. Microbiota profiles pre- and post fecal microbiota transplantation.

IS profiles of fecal samples collected pre- and post fecal microbiota transplantation (FMT) compared to donor profile. (1) pre-FMT; (2) first sample post-FMT (5 days after FMT); (3) sample collected during CDI recurrence (18 days post-FMT) prior to start of fidaxomicin; (4) sample on last day of fidaxomicin treatment (5) sample ten days after cessation of fidaxomicin, and (6) IS profile of the donor feces. Horizontal axis of each profile displays IS fragment length expressed in number of nucleotides, corresponding to bacterial operational taxonomic unit (OTU). Vertical axis of each profile displays the relative abundance of the corresponding OTU. Blue peaks represent *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* (FAFV), red peaks represent *Bacteroidetes* and yellow peaks represent *Proteobacteria*.

Microbiota composition of pre-FMT sample is characterized by presence of very limited number of species, a relatively high abundance of *Fusobacterium nucleatum* and almost complete absence of *Proteobacteria*. The patient developed a unique microbiota profile which was very stable over time despite CDI recurrence and subsequent fidaxomicin therapy. *Bacteroidetes* were stably acquired from donor feces, while FAFV and *Proteobacteria* were unique to the patient. *Clostridium difficile* could be detected in the patients profiles prior to fidaxomicin therapy, but not afterwards (abundance too low to be seen in this figure).

Remarkably, hardly any of the donor species from the phyla *Firmicutes* and *Proteobacteria* could be detected in the recipient, while a high abundance of *Fusobacterium necrophorum* and *Klebsiella oxytoca* were found in the recipient five days after FMT, both of which species were not detected preceding transplantation in the recipient or in the donor feces. We hypothesize this could be explained by two different factors. First, these species have been incorporated from the environment or family members (the patient was discharged one day after FMT). Alternatively, these species were already present in the recipient and/or donor feces prior to FMT, but in a very low abundance below detection level. Alteration of the composition and diversity of the intestinal microbiota following FMT may have provided an opportunity for *Fusobacterium necrophorum* and *Klebsiella oxytoca* to increase in abundance, leading to detectable peaks in the microbiota profiles.

C. difficile could still be detected in low abundance after FMT. Post-FMT course was characterized by a very stable course over time of species within all phyla, despite occurrence of a CDI recurrence and treatment with fidaxomicin. Interestingly, after treatment with fidaxomicin, *C. difficile* was no longer detected, while further microbiota composition remained unchanged.

DISCUSSION

Down syndrome has been associated with various immunological impairments and is linked to an increased risk for leukemia and auto-immune diseases.¹⁵ Furthermore, abnormalities in function of both innate and adaptive immunity may lead to diminished viral and bacterial clearance.¹⁶

Knowledge on characteristics of intestinal microbiota composition in Down syndrome is very limited. The largest study on this topic comprised the comparison between the

gut microbiota structure of 17 adults with Down syndrome with that of 16 healthy, non-trisomy controls by means of 454 pyrosequencing.¹⁷ Comparable levels of microbial diversity and a similar overall composition was observed in both groups. In-depth analysis showed that the gut microbiota of subjects with Down syndrome is largely dominated by the phyla *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*¹⁷, while *Proteobacteria*, *Verrucomicrobia* and *Fusobacteria* represent the more subdominant phyla.¹⁷ In our patient, *Fusobacterium nucleatum* was observed in relatively high abundance, but *Proteobacteria* were almost completely absent pre-FMT.

Data about the effectiveness of FMT in children are still scarce. To date, only a few case-reports and case-series of children treated with FMT have been described.¹⁸⁻²⁴ These studies have shown that FMT seems to be safe, well tolerated, and effective for pediatric patients with recurrent CDI, with response rates up to 95%. Case series on FMT in children include several patients receiving simultaneous immunosuppression. That these patients tolerated FMT well, indicates that an immunocompromised state is not an absolute contraindication for application of FMT in children, which is substantiated by our case.⁶

Nicholson *et al.* identified malignancy as the most important risk factor in children for both primary and recurrent CDI.² They also described an insufficient host immune response to be a causal risk factor for development of recurrent CDI. The patient described here fulfilled all these criteria; the combination of underlying Down syndrome (associated with impaired immunological function), choroid plexus carcinoma, and treatment with chemotherapy and antibiotics, have possibly contributed to the occurrence of multiple CDI recurrences. Development of a post-FMT recurrence could possibly also be directed to this extensive underlying comorbidity. It has been suggested that patients who develop a post-FMT CDI recurrence have at least a partially restored gut microbiota, which is reflected by an increased efficacy of antibiotic treatment for CDI compared to the pre-FMT state.¹¹ In

our patient, post-FMT recurrence was successfully treated with fidaxomicin, which supports this hypothesis. We preferred fidaxomicin over vancomycin, despite higher costs, because it has been shown that fidaxomicin has less negative influence on the precarious balance of the gut microbiota compared to vancomycin, sparing commensal microbiota and thus leading to a lower risk of recurrence.²⁵ This is supported by our data, where fidaxomicin did not influence the composition of the gut microbiota (Figure 1).

Conclusion

This is the first description of a child with Down syndrome treated with FMT for recurrent CDI. Our findings indicate that children with Down syndrome, characterized by an alternate immune system, may well tolerate and benefit from FMT, even in a severe immunocompromised state due to comorbid malignancy. The diversity of microbiota increased from a Shannon diversity index of 2.08 pre-FMT to 3.12 post-FMT. After FMT, the patients' microbiota profile remained very stable over time.

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